PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

SHIBUYA, *et al.*

Atty. Ref.: 249-323

Serial. No.: 10/763,276

Group Art Unit: 1642

Filed: January 26, 2004

Examiner: NICKOL, Gary B..

For: SUBSTANCE WHICH INHIBITS BINDING OF INFORMATION TRANSFER MOLECULE FOR 1175OTYROSINE PHOSPHORYLATED KDR/FLK-1 AND USAGES OF THE SAME

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Kenya SHITARA, a citizen of Japan, do hereby declare as follow:

I graduated from the University of Tokyo, Faculty of Pharmaceutical Science in 1982, entered the graduate school of the University of Tokyo immediately after graduated, and got MSc degree in 1984. My major subject in the University of Tokyo was immunology. Since 1984, I have worked at Tokyo Research Laboratories; I have been studying on establishment and evaluation of anti-tumor monoclonal antibodies. I got Ph.D. degree from the University of Tokyo, Faulty of Pharmaceutical Science in 1990. During 1993 and 1994, I stayed Neurobiology Program, La Jolla Cancer Research Foundation (Present name is The Burnham institute) U.S.A. and studied on function of the novel proteoglycan in the brain in the lab. Since 1997, I have been a senior researcher and been at the position of the head, Division of Immunology, Tokyo Research Laboratories, Kyowa Hakko Kogyo, Co., Ltd. I belong to Japanese Association

for Cancer Research, and the Japanese Association for Metastasis Research. The number of my oral and poster presentations in the academic meetings of these and other international societies, such as the International Conference on AACR-NCI-EORTC and International Symposium on Cancer Chemotherapy are more than twenty in total. My publication in academic journals are more than thirty; these journals include Journal of Biological Chemistry, Cancer Research, Journal of Immunology, Blood, Oncogene, Proceedings of national Academy of Science USA, etc.

I am familiar with the prosecution history of the above-identified patent application.

The following experimentation was conducted in order to demonstrate the unexpected effects of the present invention.

Experiment 1

Effects of KM3055, an anti- 1175 –tyrosine phosphrylated (PY1766) KDR/Flk-1 monoclonal antibody, on proliferation of human microvascular endothelial cells.

We evaluated the effect of KM3055, an anti-PY1766 KDR/Flk-1 monoclonal antibody, on proliferation of human microvascullar endothelial cells.

The monoclonal antibody KM3055 was incubated with human microvascular endothelial cells in the presence of an agent, Chariot, to incorporate

into the cultured cells in vitro. The endothelial cells were cultured 6 days and cell growth was examined. The cell growth was significantly inhibited by the treatment of the monoclonal antibody KM3055.

Material

Cells

Human microvascular endothelial cells (HMVEC) were purchased from Kurabo Industries Ltd., Japan) and cultured in HuMedia-MvG medium (Kurabo Industries Ltd., Japan) at 37°C.

Antibody and agents

KM3035 (an anti- 1175 –tyrosine phosphorylated (PY1766) KDR/Flk-1 monoclonal antibody) was established according to the specification through page 30 line 8 to page 31 line 35, and in Example 6. KM3055 antibody specifically recognized 1175-tyrosine phospholyrated KDR/Flk-1 and inhibited the binding of phospholipase C-γ (data not shown).

The following agents were purchased commercially and used: VEGF (Bio-Rad Japan, Tokyo, Japan), Chariot (Active Motif, California, USA) and MTS (Promega, Wisconsin, USA).

Methods

Treatment with monoclonal antibodies

Incorporation of KM3055 into the cells in vitro was performed according a known method as described in, for instance, Nature Biotechnology 19, 1173 (2001). KM3035 and Chariot were mixed in 1.5 ml microtube (TreffLab, Switzerland) with molar ratio 2 μM : 40 μM and incubated for 30 min at room temperature to form complex. HMVEC were seeded in 96 well plate (Asahi Techno Glass Co., Japan) at 1 x 10^5/well/100μl medium and cultured one day. After aspirating medium in the culture, 50 μl of KM3035-Chariot complex solution and 50μl of serum free medium were added and incubated for 30 min at 37°C. The medium was switched to the one containing 0.1 % serum and the cells were cultured over night to deliver KM3035 into the cell. The cells were washed with 100 μl of PBS and then cultured 6 days in the medium containing 0.1 % serum in the presence of or absence of 10 ng/ml VEGF. A rat-IgG was used as a negative control.

Measurement of cell growth

Cell growth experiments were performed according to a known method as described in, for instance, J Immunol Methods 179(1), 95 (1995). Briefly, the present experimental methods are as follows.

The cell growth was measured by MTS assay. Cells were incubated with 20 μl of MTS solution for 4 hr at 37°C, then absorbance at 490 nm was measured using Microplate Reader (Bio-Rad Japan, Tokyo, Japan).

Results

Effect of KM3055 on HMVEC cell growth

The basal cell proliferation of HMVEC was stimulated by 10 ng/ml VEGF. Treatment of KM3055 antibody significantly inhibited the cell growth driven by VEGF, compared to the control group and the rat-IgG- treated group as a negative control (Fig. 1).

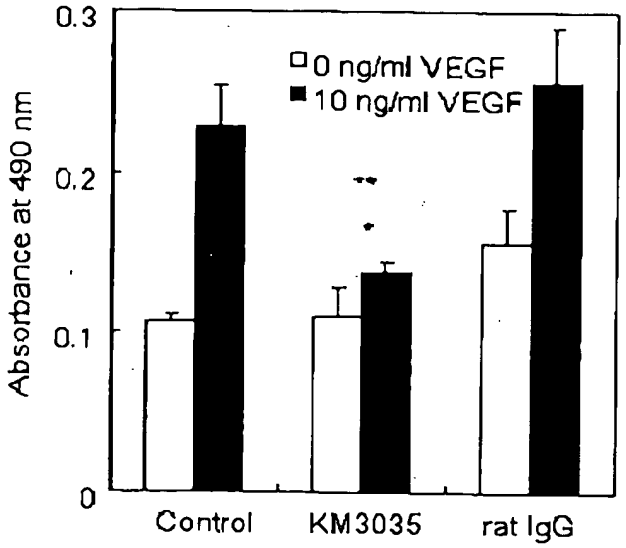

Fig.1 Effect of KM3055 on cell growth of endothelial
*: $p < 0.01$ compared to control,
**: $p < 0.05$ compared to rat IgG

U.S. APPLICATION NO. 10/763,276 PATENT APPLICATION
DECLARATION UNDER 37 C.F.R. §1.132

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: April 17, 2006 Name: Kenya SHITARA

Dr. Kenya SHITARA